

The Peptides

Analysis, Synthesis, Biology

VOLUME 2 Special Methods in Peptide
Synthesis, Part A

Edited by

ERHARD GROSS

*National Institutes of Health
Bethesda, Maryland*

JOHANNES MEIENHOFER

*Chemical Research Department
Hoffmann-La Roche Inc.
Nutley, New Jersey*

1980



ACADEMIC PRESS

A Subsidiary of Harcourt Brace Jovanovich, Publishers

New York London Toronto Sydney San Francisco

COPYRIGHT © 1979, BY ACADEMIC PRESS, INC.

ALL RIGHTS RESERVED.

NO PART OF THIS PUBLICATION MAY BE REPRODUCED OR
TRANSMITTED IN ANY FORM OR BY ANY MEANS, ELECTRONIC
OR MECHANICAL, INCLUDING PHOTOCOPY, RECORDING, OR ANY
INFORMATION STORAGE AND RETRIEVAL SYSTEM, WITHOUT
PERMISSION IN WRITING FROM THE PUBLISHER.

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data

Main entry under title:

The Peptides.

Includes bibliographical references and index.

CONTENTS: v. 1. Major methods of peptide bond
Formation.--[etc.]--v. 3. Protection of functional groups
in peptide synthesis.

1. Peptides. I. Gross, Erhard. II. Meienhofer,
Johannes. [DNLM: 1. Peptides. QU68 P424]
QP552.P4P47 574.1'9245 78-31958
ISBN 0-12-304202-X (v. 2)

PRINTED IN THE UNITED STATES OF AMERICA

79 80 81 82 9 8 7 6 5 4 3 2 1

Contents

List of Contributors	ix
Preface	xi
Nomenclature and Abbreviations	xiii
Contents of Volume 1	xix

Chapter 1 *Solid-Phase Peptide Synthesis*

George Barany and R. B. Merrifield

I	Introduction	3
II	The Solid-Phase Principle	5
III	The Anchor to the Polymeric Support	42
IV	The Repetitive Deprotection/Coupling Cycle	100
V	Protected Amino Acids	164
VI	Conclusions and Outlook	250
	Addendum	252
	References	254

Chapter 2 *The Liquid-Phase Method for Peptide Synthesis*

Manfred Mutter and Ernst Bayer

I	Introduction	286
II	Strategies of Peptide Synthesis	287
III	The Liquid-Phase Method	300
IV	Applications of the Liquid-Phase Method	321
V	Conclusions	328
	References	329

Chapter 3 Polymeric Reagents in Peptide Synthesis*Mati Fridkin*

I	Introduction	333
II	Polymeric Reagents in Organic Chemistry: General Aspects	335
III	Polymeric Reagents in Peptide Synthesis	336
IV	Concluding Remarks	359
	References	361

Chapter 4 The Four Component Synthesis*Ivar Ugi*

I	Four Component Condensation as a Basis of Peptide Synthesis	365
II	Advantages of the Four Component Synthesis	369
III	Problems	370
IV	Recent Advances	374
V	Perspectives	378
	References	379

Chapter 5 The Oxidation–Reduction Condensation*Teruaki Mukaiyama, Rei Matsueda, and Masaaki Ueki*

I	Introduction	384
II	Procedures of Peptide Bond Formation	385
III	Solid Phase Synthesis by Oxidation–Reduction Condensation	398
IV	Applications to the Synthesis of Biologically Active Peptides	408
V	Related Applications	412
VI	Concluding Remarks	414
	References	414

Chapter 6 Repetitive Methods in Solution*Lajos Kisfaludy*

I	Introduction	418
II	The Functional Handle Method	419

III	The Repetitive Excess Mixed Anhydride (REMA) Method	424
IV	Peptide Synthesis <i>in Situ</i>	429
V	The Pentafluorophenyl Ester Method	432
VI	Comparative Studies	437
VII	Concluding Remarks	438
	References	438

Chapter 7 Partial Synthesis of Peptides and Proteins

Robert C. Sheppard

I	Introduction	442
II	Chain Cleavage of Peptides and Proteins	445
III	Protection of Functional Groups	451
IV	Activation and Coupling Reactions	463
V	Selected Examples of Partial Synthesis Operations on Natural Peptides and Proteins	467
VI	Conclusions	480
	References	481

Chapter 8 Racemization and Coupling Rates of *N*^α-Protected Amino Acid and Peptide Active Esters: Predictive Potential

József Kovács

I	Introduction	486
II	Racemization of <i>N</i> ^α -Benzylloxycarbonyl and <i>N</i> ^α - <i>tert</i> -Butyloxycarbonylamino Acid Active Esters	497
III	Racemization of <i>N</i> -2-Benzylloxycarbonyl- and <i>N</i> ^α - <i>tert</i> -Butyloxycarbonylglycylamino Acid Active Esters (Dipeptides)	503
IV	Coupling Rates of <i>N</i> ^α -Alkyloxycarbonylamino Acid Active Esters	510
V	Coupling Rates of Glycylamino Acid (Dipeptide) Active Esters	518
VI	Ratio of Coupling to Racemization Rate Constants	520
VII	Relation of Coupling Time to Concentration	524
VIII	Racemization of the Penultimate Amino Acid Residue	529

IX	Prediction of Coupling and Racemization Rate Constants	530
X	Summary and Prognosis	535
	References	536
	Author Index	541
	Subject Index	567

Solid-Phase Peptide Synthesis

GEORGE BARANY and R.B. MERRIFIELD

I	Introduction	3
II.	The Solid-Phase Principle	5
A.	Generalized Solid-Phase Strategy	7
1.	The Polymeric Support	9
2.	Functionalization and Anchoring	10
3.	The Repetitive Deprotection/Coupling Cycle	10
4.	Cleavage from the Support	11
5.	Selection of the Protection Scheme	11
B.	Specific Variations of Solid-Phase Strategy	12
1.	Stepwise Condensation	12
2.	Segment Condensation	13
3.	Preparation of Protected Peptide Segments	13
4.	Side-Chain Anchoring	13
5.	Safety-Catch Anchoring	16
6.	Dual Anchoring	16
C.	Conceptual Ramifications	17
1.	Physical Chemistry of the Solid Phase	17
2.	Reaction Rates	29
3.	Elaboration of the Polymer-Bound Peptide	30
4.	Yields and Purity of Product	32
D.	Practical Ramifications	34
1.	Purity of Materials	35
2.	Mechanics	37
3.	Apparatus	38
4.	Analytical Control for Product Quality	42
III	The Anchor to the Polymeric Support	42
A.	Functionalization	42
B.	Chemistry of the Anchoring Linkage	57
1.	Substituted Benzyl Esters	59
2.	Benzhydryl Esters	84
3.	Phenacyl Esters	84

THE PEPTIDES, VOLUME 2
Copyright © 1979 by Academic Press, Inc.
All rights of reproduction in any form reserved.
ISBN 0-12-304202-X

4. Substituted Phenyl Esters	87
5. Alkyl Esters	87
6 Sulfonamides	90
7. Anchoring through Peptide Bonds	91
8. Carboxamides	93
9. Substituted Hydrazides	96
10. Substituted Urethanes	97
11. Dinitrophenylene Linkages	99
IV. The Repetitive Deprotection/Coupling Cycle	100
A. Temporary Protecting Groups	100
1. <i>tert</i> -Alkyl Urethanes Removable by Acidolysis	102
2. Substituted Arylisopropyl Urethanes Removable by Acidolysis	104
3. Other Amino Protecting Groups Removable by Acidolysis	106
4. Arylsulfonyl Groups Removable by Acidolysis and Nucleophilic Cleavage	109
5. Urethanes Removable under Basic Conditions	112
6. Dithiasuccinoyl Group Removable by Reduction	114
7. Miscellaneous Amino Protecting Groups	116
8. Carboxyl or Hydrazide Protecting Groups	118
B. Neutralization	118
C. Formation of the Peptide Bond	122
1. Carbodiimides	123
2. Symmetrical Anhydrides	137
3. Active Esters	139
4. Oxidation-Reduction Condensation	142
5. Other Coupling Methods and Reagents	144
D Monitoring	149
1. Experimental Approaches	149
2. Quantitation	155
3. Evaluation of Reaction Endpoints	155
4. Analysis of Deletion Peptides	156
5. Radiolabeled Peptides	158
E Systematic Elimination of Deletion and Terminated Peptides	159
1. Blocking of Unreacted Amino Groups to Suppress Formation of Deletion Peptides	159
2. Affinity Purification to Remove Terminated Peptides	163
V Protected Amino Acids	166
A. Chemistry of the Protection Scheme	166
B. Chemistry of the Individual Amino Acid Residues	169
1. Arginine	169
2. Lysine and Ornithine	175
3. Histidine	179
4. Aspartic and Glutamic Acid	190
5. Asparagine and Glutamine	199
6. Serine and Threonine	208
7. Tyrosine	211
8. Phenylalanine	217
9 Tryptophan	217

10. Methionine	223
11. Cysteine	233
12. Proline and <i>N</i> -Methylamino Acids	247
13. Glycine, Alanine, Valine, Leucine, Isoleucine, and Norleucine	249
VI. Conclusions and Outlook	251
Addendum	252
References	255

I. INTRODUCTION

The science and art of peptide synthesis was founded at the beginning of the twentieth century by Emil Fischer and Theodor Curtius, and has steadily been developed into a discipline of great power and sophistication. The classical methodology has been reviewed by Greenstein and Winitz (1961), Schröder and Lübke (1965), Wünsch (1974), Bodanszky *et al.* (1976), Finn and Hofmann (1976), and in these volumes of "The Peptides." However, as recently as 15 years ago, the successful synthesis of biologically active materials still required considerable investments of time, effort, and manpower. Perhaps the most intimidating aspect of the field has been the need to purify, by discriminating methods, all intermediates of lengthy multistep routes, a non-routine and demanding task. The problem is made all the more challenging by the unpredictable solubility characteristics of the various intermediates.

Solid-phase peptide synthesis was conceived in 1959, at a time when chromatography on insoluble resins was being applied with great success to the analysis of amino acids, peptides, and proteins. It was reasoned that by attaching a growing peptide chain to an insoluble polymeric support, excess reagents and by-products from the synthetic cycles could be removed by simple filtration and washing steps. Thus, the purification problem of classical methods of peptide synthesis ought to be readily circumvented, while most of the highly honed chemistry of coupling and functional group protection was expected to be readily adaptable to the new strategy. As an added benefit, the entire solid-phase procedure was predicted to be amenable to automation. The feasibility of these ideas was first shown by the synthesis of the crystalline tetrapeptide L-leucyl-L-alanyl-glycyl-L-valine (Merrifield, 1962, 1963). At about the same time, Letsinger and Kornet (1963) reported the polymer-supported synthesis of L-leucyl-glycine, using a different chemical approach. Further developments followed soon thereafter in several laboratories, and a useful and practical set of methods has evolved that will be the subject of this review.